PATENT
Docket No. 475512000400

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:		Examiner:	S. Saucier
	Steven M. WATKINS	Group Art Unit:	1651
Serial No.:	10/808,880		
Filing Date:	March 24, 2004		
For:	METHODS OF USING QUANTITATIVE LIPID METABOLOME DATA		

DECLARATION OF STEVEN M. WATKINS, PH.D.
PURSUANT TO 37 C.F.R § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Steven M. Watkins, Ph.D., declare as follows:

1. I am currently President and Chief Scientific Officer of Lipomics Technologies. My Curriculum Vitae is attached hereto as Exhibit 1.

2. I am an inventor of the above-identified patent application and am familiar with its contents and the pending claims.

3. I am also familiar with the non-final Office Action mailed December 27, 2006 in the above-identified patent application.

4. Experiments presented in Example 1 in the specification of the above-identified application and additional experiments of which I am aware (i.e., Studies A-D, summarized below) show that there is a correlation between (a) palmitoleic acid (16:1n7) and/or the ratio of palmitoleic acid (16:1n7) to palmitic acid (16:0) in blood and (b) weight gain/loss or *de novo*

fatty acid synthesis in adipose or the liver. These experiments support the assertion that either palmitoleic acid (16:1n7) and/or the ratio of palmitoleic acid (16:1n7) to palmitic acid (16:0) in blood can be used as markers for the assessment of (a) *de novo* fatty acid synthesis in adipose or the liver and/or (b) a propensity for weight gain/loss.

5. Example 1 of U.S. Serial No. 10/808,880. Example 1 at pages 38-62 of the specification of U.S. Serial No. 10/808,880 provides data that shows a correlation between the level of palmitoleic acid (16:1n7), as well as the ratio of palmitoleic acid (16:1n7) to palmitic acid (16:0), in certain lipid classes with *de novo* fatty acid synthesis in adipose and the liver, as well as with weight gain/loss.

6. Example 1 reports the findings of a study in which the lipid composition in various tissues and plasma was measured in prediabetic mice (a cross of NZO and NON mouse strains) that had been treated with rosiglitazone, in NON mice that had been treated with CL316,243, and in control mice. Rosiglitazone is known to increase *de novo* fatty acid synthesis in adipose and the liver. See, e.g., McTernan, et al., *Diabetes* 51(5):1493-8, 2002, Patel et al., *Diabetes* 52(1):43-50, 2003, and Edvardsson et al., *Proteomics* 3(5):803-4, 2203. CL316,243, on the other hand, has been shown to be linked to decreased lipogenesis. See, e.g., Ferrand et al., *J. Physiol. Biochem.* 62(2):89-99, 2006. The results of the study in Example 1 are shown in Tables 5-8 and described on pages 60-62 of the specification. As indicated on page 60 of the specification, in Tables 5 and 7, the lipid composition data are expressed as nanomoles per gram of tissue or plasma, and in Tables 6 and 8, the lipid composition data are expressed as a percentage of total fatty acids within each lipid class.

7. The lipid composition data in Tables 5-6 at pages 46-47 and 49-50 of the specification show that when rosiglitazone (i.e., a treatment known to increase *de novo* fatty acid synthesis) was given to the mice, the amount of palmitoleic acid in cholesterol esters in plasma, the amount of palmitoleic acid in phosphatidylcholines in plasma, and the amount of palmitoleic acid in free fatty acids in plasma all increased relative to control. The data in Tables 5-6 at pages 46-47 and 49-50 of the specification also show that when rosiglitazone was given to the mice, the ratio of palmitoleic acid to palmitic acid in cholesterol esters in plasma, the ratio of palmitoleic acid to palmitic acid in phosphatidylcholines in plasma, and the ratio of palmitoleic acid to palmitic acid in free fatty acids in plasma all increased relative to control.

8. As further indicated in Example 1 at page 61, lines 14-16, and Tables 7 and 8 of the specification, treatment of mice with CL316,243, on the other hand, induced a substantial decrease in palmitoleic acid concentrations in most lipid classes in heart, liver, adipose, and plasma. The lipid composition data in Tables 7 and 8 at pages 52, 58-59 of the specification show that when CL316,243 was administered to the mice, decreases in the level of palmitoleic acid, as well as in the ratio of palmitoleic acid to palmitic acid, in phosphatidylcholines in plasma, free fatty acids in plasma, and cholesterol esters in plasma relative to the controls were observed.

9. In addition to measuring the lipid compositions, the body weight gain and adipose tissue weight gain of the mice in the study in Example 1 were also measured. The mice in the study that were given rosiglitazone showed a "significant increase" in both total body and adipose tissue weight, as stated in lines 14-16 of page 60 of the specification, whereas mice in the study that were given CL316,243 showed a "significant decrease" in both total body and adipose tissue weight as clearly stated in lines 17-19 of page 61 of the specification and shown in Figures 2 and 3. (The descriptions of these figures on pages 5-6 of the specification contain errors.) Furthermore, as indicated at lines 11-13 of page 61 of the specification, clinical studies in humans have shown that patients taking rosiglitazone gain weight (Füchtenbusch et al., *Exp. Clin. Endocrinol. Diabetes* 108:151-163, 2000).

10. Thus, the experiments described in Example 1 of the specification demonstrate that both palmitoleic acid and the ratio of palmitoleic acid to palmitic acid in certain lipid classes (e.g., cholesterol esters, phosphatidylcholines, and free fatty acids) in plasma positively correlate with rosiglitazone treatment, which in turn, positively correlates with *de novo* fatty acid synthesis in both adipose and the liver and correlates with weight gain. The experiments described in Example 1 also demonstrate that a decrease in the level of palmitoleic acid or in the ratio of palmitoleic acid to palmitic acid in certain lipid classes (e.g., cholesterol esters, phosphatidylcholines, and free fatty acids) in plasma correlates with CL316,243 treatment, which in turn, correlates with decreased *de novo* fatty acid synthesis and with weight gain. These data support the claim that both palmitoleic acid and the ratio of palmitoleic acid to palmitic acid in certain lipid classes in plasma can be used as markers for *de novo* fatty acid synthesis in adipose and the liver and for a propensity for weight gain/loss.

11. Additional Data (Studies A-D). In addition to the data provided in Example 1 of the application as filed, additional experiments that have been conducted by and/or at Lipomics Technologies further support the feasibility of using the level of palmitoleic acid and the ratio of palmitoleic acid to palmitic acid in cholesterol esters in plasma as markers for *de novo* fatty acid synthesis and weight gain/loss. These experiments, Studies A-D, and their results are briefly summarized below.

12. Study A: Preclinical data has been obtained that demonstrates the positive correlation of both (a) palmitoleic acid in cholesterol esters (CE16:1n7) in plasma and (b) the ratio of palmitoleic acid to palmitic acid in cholesterol esters (CE16:1n7/CE16:0) in plasma to the expression of fatty acid synthase (FAS). FAS is a cytoplasmic enzyme complex that catalyzes the synthesis of long-chain saturated acyl-CoAs (fatty acids) from acetyl-CoA and malonyl-CoA. This activity is the first step in the synthesis of fatty acids and *de novo* lipogenesis.

13. In Study A, two different genotypes and four different drugs were tested on mice. The genotypes tested were db/+, a healthy phenotype and db/db, a leptin receptor knock out. Within each genotype, four treatments were administered with 10 mice per treatment group. The treatments were vehicle and rosiglitazone at low, mid, and high dose. Serum, liver, muscle and adipose were collected from the mice. Serum samples were analyzed, and fatty acid compositions of lipid classes were measured. Data were also obtained from expression profiles performed on the liver of each mouse, including the expression of FAS.

14. The results of Study A are shown in Exhibit 2. The leptin receptor deficiency did not significantly affect baseline FAS expression as assessed by a Student's t-test (alpha< 0.05). The leptin receptor deficiency strongly influenced model response to rosiglitazone (RG), causing substantially increased FAS in response to treatment. The increased FAS was not observed in the normal (db/+) mice, indicating a qualitative difference in drug response between diseased and normal animals. As shown in Exhibit 2, the increase in FAS expression was mirrored by plasma concentrations of CE16:1n7 and by the ratio of the amount of CE16:1n7 to CE16:0 in plasma in leptin deficient mice. Thus, both the level of CE16:1n7 and the ratio of CE16:1n7 to CE16:0 in plasma provide a good indication from plasma that fatty acid synthesis in the liver is increased.

15. Data obtained in further additional studies, Studies B-E, summarized below, demonstrate the correlation of (a) palmitoleic acid and (b) the ratio of palmitoleic acid to palmitic acid in cholesterol esters in plasma to weight gain or loss.

16. Study B: Study B study showed that CE16:1n7 and the ratio of CE16:1n7 to CE16:0 in plasma were correlated with weight gain induced by treatment with a PPARgamma agonist (other than rosiglitazone). It is well known that PPARgamma agonists cause weight gain specifically caused by deposition of fat in adipose (see, e.g., Nichols et al., *Diabetes Obes. Metab.* 9(1):96-102, 2007). Given the correlation of CE16:1n7 and the ratio of CE16:1n7 to CE16:0 with FAS expression (see the description of Study A, above), the results of Study B indicate that *de novo* fatty acid synthesis results in weight gain and can be observed in plasma by measuring specific fatty acids.

17. In Study B, four different treatments were given to 24 ZDF rats, with six rats per treatment group. Treatments included vehicle, a PPARalpha agonist, a PPARdelta agonist, and a PPARgamma agonist. Serum samples were collected after a certain period of time after treatment and the fatty acid profiles were measured by Lipomics Technologies. Weight gain was carefully measured in each rat from pre-treatment to post-treatment.

18. The results of Study B are shown in Exhibit 3. Comparison of weight gain between treatment groups found that the PPARg-treated rats gained significantly more weight than all other groups. The level of plasma CE16:1n7 correlates strongly with the amount of weight gain with an R-squared value of 0.936. The ratio of CE16:1n7 to CE16:0 also had a strong positive correlation with body weight gain; the R-squared value was 0.9 with a p-value less than 0.0001.

19. Study C. Study C provided data indicating that CE16:1n7 and CE16:1n7/CE16:0 correlated with rosiglitazone treatment in humans. The study examined the effect of rosiglitazone, metformin, and glyburide treatment relative to placebo in mildly diabetic patients.

20. In Study C, mild diabetic subjects not taking any medication were treated with one of four compounds for four weeks. Nineteen subjects were treated with metformin, 20 with glyburide, 20 with rosiglitazone, and 15 with a placebo. Serum, muscle, and adipose were collected at baseline, 2 weeks post-treatment and 4 weeks post-treatment. Body weights were also taken at baseline, 2, and 4 weeks post-treatment.

21. Some of the results of Study C are shown in Exhibit 4. Exhibit 4 shows the percentage change in levels of CE16:1n7 and in CE16:1n7/CE16:0 in the serum of subjects treated with rosiglitazone. CE16:1n7 and CE16:1n7/CE16:0 were found to be significantly increased from baseline to two and four weeks post-treatment in the rosiglitazone-treated group while no significant changes were observed in the other groups, including placebo. Although weight gain data was not obtained for this study, subjects taking rosiglitazone are known to gain weight as fat, and CE16:1n7 and the ratio of CE16:1n7 to CE16:0 indicated they increased their synthesis of fat in response to treatment.

22. Study D. An additional study, Study D, provided data that serum levels of CE16:1n7 and CE16:1n7/CE16:0, markers which correlate with *de novo* fatty acid synthesis, were reduced during caloric restriction and weight loss.

23. In Study D, two independent cohorts of subjects undergoing a severe caloric restriction for six weeks were profiled. Body weights and metabolites in both studies were measured at three time points: Day 1, Week 3, and Week 6. The first cohort had 23 subjects with 20 females and 3 males, and the second cohort had 27 subjects with 18 females and 9 males.

24. The results of Study D are shown in Exhibit 5. All subjects in the study lost weight over the time course. Exhibit 5 shows the percent change from day 1 in the level of CE16:1n7 and CE16:1n7/CE16:0 in the cohorts at week 3 and week 6. Caloric restriction caused a large and significant decrease in CE16:1n7 and in the ratio of CE16:1n7 to CE16:0 in serum cholesterol ester pools in both cohorts, consistent with a general down regulation of hepatic *de novo* lipogenesis. The decreased level of CE16:1n7 and a decreased ratio of CE16:1n7 to CE16:0 correlated with weight loss in both cohorts.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

6/26/07
Date

Steven M. Watkins, Ph.D.

Exhibit 1. Curriculum Vitae
Exhibit 2. Study A results
Exhibit 3. Study B results
Exhibit 4. Study C results
Exhibit 5. Study D results